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History. In August, 1895, we received a glass jar containing a small quantity of ice-cream which had poisoned a number of people at a small village in Northern Michigan. In October of the same year Dr. Morris, of Vassar, Michigan, sent to us a small portion of cheese which had caused alarming illness in a number of people of that place. These samples of suspected food were examined by the method which we employ in such cases at the Laboratory of Hygiene of the University of Michigan, and which we have published elsewhere. The poison-producing germ was found to be the same in the two articles of food, a fact which was suggested by the similarity in the symptoms observed and reported by the attending physicians at the two places.

Symptoms. Some fifty people partook of the cream, and all were more or less seriously affected. The number known to have suffered after eating the cheese was twelve. There were no deaths. The symptoms appeared from three to six hours after the food was eaten. The first evidence of illness consisted of nausea, which in all instances was followed by vomiting. Diarrhœa was present in the majority, but not in all. The vomiting was accompanied by sharp pains through the abdomen, and it is stated that in some the pain was partially relieved by strong pressure. The most alarming phenomenon observed by the physicians in attendance was feebleness of the heart's action. The hands and feet grew cold, then the entire body became cool and clammy, and in many the radial pulse was not perceptible. This con-

dition, together with a heavy stupor in some, gave occasion for alarm to the attending physicians, and hypodermic injections of brandy, digitalis, strychnin and nitroglycerin were employed, each physician selecting the stimulant in which he had most confidence, or taking that which he had at hand. In some, the pupils were said to be dilated, but the evidence on this point is confined to the testimony of one physician. In one instance the patient became wildly delirious, crying out, and attempting to rise from bed. Those who vomited but little and had no diarrhœa fell into a heavy stupor, and it is highly probable that these were in greater jeopardy than any of the others. The early and thorough vomiting doubtlessly was the most potent agent in saving those who had taken the larger quantities of the infected food. As has been stated, the depressing action of the poison on the heart impressed the physicians in attendance so markedly that all mention it, and one who had seen other cases of cheese-poisoning thought that the active agent in this instance must differ from that which had caused the sickness previously observed by him.

Morphology of the Germ. The infecting organism in the ice-cream and cheese is a bacillus, which grows readily both in aërobic and anaërobic cultures. The form of the bacillus is subject to some variations, dependent upon the medium on which and the conditions under which it is grown. Usually it forms rods the length of which is from two to three times its breadth. Under most conditions the rods are single, but at times an end-to-end growth of from two to four bacilli may be seen. Threads are sometimes formed, and in other instances the coccus-form may be approached, but never reached. Germs taken from an agar-tube, after twenty-four hours' growth in the incubator, gave an average length of 1.72μ and a breadth of 0.86μ. The formation of spores has not been observed.

Behavior with staining reagents. Germs taken from agar-tubes after eight days' growth in the incubator fail to stain with methylene-blue, even after being heated or after being left in the stain at ordinary temperature for five minutes, but do stain readily with carbolic fuchsin. Preparations made from the tissues and fluids of the bodies of animals dead from the effects of the germs take all the basic anilin dyes readily. By Gram's method the bacillus is decolorized. In staining tissue containing the germ the best results are obtained with Löffler's methylene-blue and a contrast with eosin.

Motility. From tubes grown in the incubator this bacillus shows marked motility, which is, however, much less noticeable in cultures grown at ordinary temperature.

Growth on gelatin. Stab-cultures in gelatin show a continuous growth along the needle track, and spread slightly about the point of inoculation on the surface. There is no liquefaction, and after twenty-four hours or longer one or more gas-bubbles may be seen along the line of growth. The surface growth is white, while that along the line as seen through the gelatin is yellowish. "Shake" tubes in gelatin begin to grow cloudy after from sixteen to twenty-four hours, and later numerous small gas-bubbles form. These bubbles grow larger for a few days and then gradually disappear. On gelatin plates the colonies show considerable variety of form; many are round, others are oval, and some quite irregular in outline. The superficial colonies appear granular and spread about a more dense nucleus. Blue litmus gelatin soon becomes red and cloudy; later, the color wholly disappears.

Growth on agar. On ordinary agar in inclined tubes the growth spreads over the surface, and has a white, slightly glazed appearance. Stab-cultures grow well along the line and spread over the surface. Glucose agar cultures show abundant white growths, spreading over the surface and producing gas in the deeper layers. Glycerin agar-tubes are similar to those of glucose agar, with the exception of the fact that no gas develops in the former.

Growth in beef-tea. Beef-tea cultures grown at 37° become cloudy after about twelve hours, and later a pellicle forms, and through this gas-bubbles may be seen arising. After from three to four days the bacterial growth subsides, leaving the supernatant fluid quite clear. Glucose beef-tea-tubes evolve larger quantities of gas.

Growth in milk. Milk is coagulated by this bacillus within from twelve to fourteen hours, when kept at 37°. Later coagulation becomes complete, and the fluid separates into a coagulum and a whey. Milk cultures soon develop a pleasant odor of butyric ether, and this persists so long as the culture remains uncontaminated. The development of acid is accompanied by the liberation of gas. This continues until all the milk sugar is consumed, and for this a period of about one month in litre flasks is required. Milk rendered feebly red with rosolic acid is decolorized after two or three days in the incubator.

This bacillus decomposes glucose, lactose, sucrose, maltose, dextrin, starch, and glycogen.

On blood-serum. On this medium our bacillus forms a thin white or yellowish growth.

On potato. Our bacillus forms a yellowish, thick, slimy, raised growth and develops a sour odor. This growth is the same whether the surface of the potato be naturally acid or alkaline, or be rendered feebly alkaline with sodium carbonate.

Growth on other vegetables and fruits. This bacillus grows well on turnip, beet, sweet potato, onion, parsnip, carrot, banana, and apple. The growth on turnip is abundant, grayish, and moist; on beet, white and glazed; on sweet potato, dry and white; on onion, white and slimy; on parsnip, yellowish, and evolving much gas; on carrot, an abundant, raised creamy growth, of sour odor; on banana, slightly elevated and slimy; on apple, thin and white. Cultures of this bacillus on these fruits and vegetables were made on account of the well-known observation that milk kept near decomposing fruits and vegetables frequently causes unpleasant symptoms in those drinking it.

Growth in Uschinsky's fluid. A culture medium free from proteid material was employed. The following, which is one of Uschinsky's formulæ, was selected:

Glycerin	40 parts.
Sodium chloride	7 "
Calcium chloride	0.1 part.
Magnesium sulphate	0.4 "
Dipotassium hydric phosphate	2.5 parts.
Ammonium lactate	6 "
Sodium asparginate	3.4 "
Water	1000 "

In this fluid our bacillus grows abundantly, and these cultures were utilized, as will be seen later, in the study of the chemical poisons produced by the bacillus. One interesting fact was observed in this connection, and, if properly interpreted by us, it shows that the presence of potassium in the culture medium is a necessity for the growth of this bacillus. When disodic hydric phosphate was substituted for the corresponding potassium salt in the above formula, the germ failed to grow and the fluid became sterile. This was tried in six flasks, and there was absolutely no growth in any of them. The only alteration

in the fluid was the substitution of the sodium for the potassium salt.

Effects of temperature on growth. This germ finds its optimum temperature at about 38°. However, it develops well at any point between this and 25°. Below the last-mentioned temperature growth is slow, or fails altogether. Beef-tea cultures were kept frozen for twenty-nine days without destroying the vitality of the bacillus. Alternate freezing and thawing carried through the same time likewise failed to kill.

Twenty beef-tea-tubes were inoculated with this germ, and kept in an air-bath at from 40° to 45°, and one tube was removed at the expiration of each hour and placed in the incubator at 37°. All of these tubes developed, thus showing that an exposure of twenty hours at this temperature does not kill. A temperature of from 45° to 50° maintained for thirty-one hours failed to kill, while the same temperature for forty-seven hours did kill. Another experiment showed that exposure to the last-mentioned temperature for thirty-five hours did destroy the germ.

A beef-tea culture which had been growing for twenty-four hours in the incubator at 37° was placed in an air-bath, kept at 54°. A loop of this culture was removed at the expiration of each hour, and a gelatin plate made from it. The number of germs on each plate was counted after twenty-four hours, with the following results: One-hour plate, 207 colonies; two-hour, 156; three-hour, 91; fourth-hour, 54; fifth-hour, 25; sixth-hour, 3; seventh-hour, 3; eighth-, ninth-, and tenth-hour, no growth.

Thirty beef-tea-tubes were inoculated and placed in a steam sterilizer. These tubes were removed at intervals of one minute, and immediately placed in the incubator at 37°. The only tube which showed any development was that removed after an exposure of one minute.

The thermal death-point, as determined by Sternberg's method, was found to be 58°.

Effects of mercuric chloride. Ten silk threads, which had been saturated with a beef-tea culture of the bacillus, and then dried in sterilized dishes at room-temperature, were placed in a 1 : 1000 solution of mercuric chloride. Six of these threads were removed at intervals of one minute, placed in beef-tea-tubes, and kept in the incubator. The one that had been exposed to the mercuric chloride for only one minute showed a good growth after eighteen hours; the others,

not until thirty-six hours. Of the four other threads, one was removed after eight, one after ten, one after fifteen, and one after twenty minutes. The beef-tea-tubes in which these were placed remained sterile.

Eight threads were placed in a 1 : 5000 solution of mercuric chloride. The first was removed after two ; the second, after five ; the third, after eleven ; the fourth, after fifteen ; the fifth, after twenty ; the sixth, after thirty ; the seventh, after forty ; the eighth, after sixty minutes. All of these were placed in beef-tea-tubes and kept at 37° in the incubator. The first three showed a good growth after eighteen hours ; the fourth only after forty hours ; while the others failed to show any development. A similar experiment was made with a 1 : 20,000 solution of mercuric chloride, but all the threads developed in the beef-tea.

Effects of carbolic acid. Threads similar to those employed with the mercuric chloride were sterilized on contact with 5, 4, and 3 per cent. solutions of carbolic acid ; after two minutes, with a 2 per cent. solution, and after ten minutes with a 1 per cent. solution.

Differentiation from the bacillus coli communis. This bacillus was at first suspected of belonging to the colon group, and comparison-tests were made between the two. The most important results obtained in this comparison may be stated as follows : (1) The new bacillus fails to give the indol reaction. (2) Both coagulate milk, but the new germ acts more promptly than the colon bacillus. It may be stated here, parenthetically, that the colon bacilli used in this comparison were from two sources, one culture having been obtained some years ago from the laboratory of the Hygienic Institute at Berlin, while the other was separated from feces for the purpose of this comparison. (3) The pleasant butyric ether odor of milk cultures of the ice-cream bacillus is not developed in cultures of the colon bacillus in the same medium. (4) The new germ grows abundantly on carrots, forming a raised, creamy layer, and gives off a sour odor ; while the colon bacillus grows much less vigorously and gives off no similar odor. On turnip, the new germ grows vigorously, forming a thick grayish layer, and this also develops a sour odor ; while on the same medium the colon bacillus develops relatively feebly. On banana, onion, parsnip, and apple the new bacillus grows much more abundantly than does the colon bacillus. (5) Milk colored with rosolic acid

is decolorized much more quickly by the ice-cream bacillus than by the colon germ.

PATHOGENESIS. This germ is pathogenic to guinea-pigs, rabbits, cats, dogs, mice, and rats. Its virulence is increased by being carried through animals. In one series we employed fifty-one guinea-pigs, inoculating each with a culture made from the preceding animal. In all of this series the inoculations were made intra-abdominally. Of the culture with which we began, one cubic centimetre of a beef-tea growth twenty-four hours old was necessary in order to kill a guinea-pig of from two to three hundred grams within twenty-four hours; while of the cultures made from the animals near the end of the series, one-fiftieth of a cubic centimetre of like growth produced the same result. The decrease in the virulence of the germ when grown on the ordinary culture media is rapid, and the intensified virulence attained in the series referred to above disappeared in the third or fourth generation when grown on gelatin or agar. Milk seems to be the most suitable culture medium. We do not know that the germ multiplies more rapidly in milk than it does in beef-tea, but cultures in the former are more virulent than those in the latter. The suspension of the germ in sterilized milk when the inoculation is made renders its action more certain. One-fiftieth of a cubic centimetre of a beef-tea growth of our intensified germ added to one cubic centimetre of milk and immediately injected into the abdominal cavity of a half-grown guinea-pig invariably caused death within twenty-four hours; while an equal amount of the same culture added to beef-tea and injected into companion animals caused death only after a much longer period, and in some failed wholly to do so.

The germ taken from the exudate in the abdominal cavity and used directly for the inoculation of another animal is more virulent than if it be carried through a culture medium before the inoculation is made. The number of germs in one cubic centimetre of such a peritoneal exudate was determined in one instance and found to be 34,800,000. One one-hundredth of a cubic centimetre of this fluid injected into the abdominal cavity of a guinea-pig weighing 350 grams caused death within twenty hours, while one-half of this quantity failed to cause any visible effects. It will, therefore, be seen that the number of germs in the most virulent culture necessary to kill a half-grown guinea-pig, when injected intra-abdominally, is somewhere between

348,000 and half that number. Subcutaneously larger amounts of the cultures were necessary to cause death.

Experiments with Guinea-pigs. A few extracts from our record book will illustrate the action on guinea-pigs.

Aug. 1, 1895. Guinea-pigs Nos. 1 and 2 were treated intra-abdominally, each with one cubic centimetre of a beef-tea culture twenty-four hours old. The injection was made at 6 P.M. Both pigs were found dead at 7 o'clock the next morning. There was some gas in the subcutaneous tissue. The abdominal muscles were congested. The peritoneum was dotted with hemorrhagic spots. The cavity was filled with a reddish fluid. The liver was covered with a plastic exudate. The thoracic cavity contained some reddish exudate. The heart was in diastole and filled with blood. Cover-glass preparations made from the subcutaneous tissue, the peritoneum, the exudates of both cavities, and the blood of the liver, spleen, and heart showed the presence of the germ in pure culture.

Striking illustrations of phagocytic action may be obtained by injecting cultures, which are not sufficiently virulent to kill, into the abdominal cavity and killing the animal within the next two days. The following is an example: Guinea-pig No. 23, weight 233, was given 0.5 of a cubic centimetre of a beef-tea culture at 4 P.M., October 21, 1895. The weight, twenty-four hours later, had fallen to 214, and at the expiration of forty-eight hours to 198. The animal was killed at 4 P.M., October 23d. The muscles about the place of injection were highly inflamed. There was some wine-colored fluid under the skin. Germs were abundant in this fluid and in smears made from the subcutaneous tissue. Some of these preparations showed phagocytes filled with bacilli, but the most beautiful demonstrations of phagocytic action were found in smear preparations from the peritoneum. In these the phagocytes were innumerable, and many of them were crowded with bacilli.

The effects of a subcutaneous inoculation with a highly virulent culture is illustrated in the following: Guinea-pig No. 33, weight 207, received subcutaneously on the back one cubic centimetre of the fluid from the peritoneal cavity of No. 32 at 8.30 A.M., October 26, 1895. By 2 P.M. of the same day the weight had fallen to 200, at 10 A.M., October 27th, it was 188, and after death, at 2 P.M. of this day, the weight was 185. There was profuse diarrhœa during

the last twenty-four hours of life. The examination showed marked inflammation and infiltration subcutaneously over the entire trunk, under the skin of the abdomen, as well as under that of the back where the injection was made. Germs were found to be abundant both subcutaneously and within the peritoneal cavity.

The amount of fluid exudate found in the peritoneal cavity after both subcutaneous and intra-abdominal inoculations has been observed to be very variable. Guinea-pig No. 46, weight 230 grams, had, at 4 P.M., November 5th, intra-abdominally, one cubic centimetre of a milk culture six days old. It was found dead at 7 A.M., November 6th, and evidently death had occurred some hours before. The subcutaneous bloodvessels were much ingested, and we took from the peritoneal cavity seven cubic centimetres of a wine-colored fluid.

In some instances the peritoneal cavity has been found to be free from fluid.

Guinea-pigs have not been inoculated with this bacillus otherwise than subcutaneously and intra-abdominally.

Experiments with Cats. The following are some of our experiments made upon cats:

Cat No. 2 was inoculated intra-abdominally with 0.5 cubic centimetre of fluid taken from the peritoneal cavity of guinea-pig No. 11. The injection was made 3 P.M., October 12th. After one and one-half hours a profuse diarrhœa began and continued until death, which resulted at 11.20 A.M., October 13th. Examination showed marked inflammation of the peritoneum. There were no gross changes observable in the intestines.

Cat No. 6 had intra-abdominally, at 4 P.M., October 26th, one cubic centimetre of fluid from the peritoneal cavity of guinea-pig No. 31. The animal was not seen again until 8.30 A.M., October 27th, when it was found in collapse with a watery diarrhœa which had evidently been established some hours before. Death followed two hours later. The condition of the body was the same as that found in cat No. 2.

Cat No. 8 had intra-abdominally two and one-half cubic centimetres of fluid from the peritoneal cavity of guinea-pig No. 40 at 5 P.M., November 1st. Four hours later this animal was found to be vomiting and purging most profusely. It was dead at 9 A.M., November 2d. There was no inflammation at the point of inoculation

or in the peritoneum. Four cubic centimetres of fluid were taken from the peritoneal cavity. The heart was in diastole and filled with blood.

These cases show that when the inoculation is made intra-abdominally the action of the germ on the cat is prompt and vigorous. All the cats used were full grown.

Large quantities of cultures given by the mouth were without effect upon cats, as is illustrated by the following:

Gave to cat No. 6, through a stomach-tube, twenty-five cubic centimetres of a beef-tea culture nine days old. There was not the slightest evidence of the animal being in any way affected. Later this animal was inoculated intra-abdominally with the result already stated.

Cat No. 4 had by stomach fifty cubic centimetres of a beef-tea culture four days old; no effect.

Cat No. 5 had by stomach fifty cubic centimetres of an Uschinsky culture; no effect.

These results are interesting and are confirmatory of some experiments made by one of us some years ago in the study of certain samples of poisonous cheese. The smallest bit of this cheese induced vomiting in man, but a cat was kept for days with no other food than the cheese; she ate freely of this and was not affected thereby. We regret that we could find no young kittens upon which these experiments might be repeated.

Subcutaneously as much as four cubic centimetres of fluid from the peritoneal cavity of a guinea-pig, dead from the action of the germ, caused only a temporary local inflammation in the cat.

Two cubic centimetres or more of a beef-tea culture injected into the jugular vein in cats caused diarrhœa, prostration, and death.

Experiments with Rabbits. The following extracts from our record book illustrate the action of this bacillus on rabbits:

Rabbit No. 15 had injected into the jugular vein four cubic centimetres of fluid from the peritoneal cavity of guinea-pig No. 35. Death resulted five hours later. Post-mortem showed no fluid in the peritoneal cavity. The capsule of each kidney was distended like a bladder around the organ. Kidneys and liver were very soft and puffy. Smear preparations made from these organs showed myriads of germs. The heart was in diastole and filled with blood. The

pericardium contained some gas. It should be stated that this examination was not made for some hours after death, but the body lay during this time in the ice-box.

The above shows the effects of an intravenous injection of a large amount of a most virulent culture. With smaller quantities of ordinary beef-tea cultures the results are not so striking.

Rabbits Nos. 81, 82, 83, and 84 had each intravenously two cubic centimetres of a beef-tea culture twenty-four hours old. Nos. 81 and 84 died about twenty hours later, while the other two, although evidently quite sick for some days, ultimately recovered. In the examination of the dead ones the surface of the kidneys, on removing the capsule, was found in all to be dotted with hemorrhagic spots. The heart was in diastole and filled with blood, but neither the pericardium nor the renal capsules contained gas.

Intra-abdominally a virulent culture of the germ affects rabbits quite as promptly and seriously as it does cats. Rabbit No. 3 had 0.2 cubic centimetre of fluid from the abdominal cavity of guinea-pig No. 9. The rabbit was found dead twelve hours later, and evidently it had been dead for some hours. The subcutaneous bloodvessels were engorged. The peritoneum was only slightly inflamed, and the cavity contained four cubic centimetres of fluid.

In several instances we have seen rabbits lie for hours after an abdominal inoculation, in an apparently moribund condition, and then slowly recover. Repeatedly the janitor has reported the animal, either rabbit or guinea-pig, in a certain cage dead, and so it was apparently, but close examination has shown a faint motion of the heart, and in some cases, after the animal has remained in this condition for twenty-four hours, or even longer, it has slowly recovered.

Experiments with Rats. White rats succumb to both subcutaneous and intra-abdominal inoculations of from one to two cubic centimetres of a beef-tea culture. The post-mortem conditions are the same as those observed in guinea-pigs.

Experiments with Mice. White mice have not yet been tested with any thoroughness so far as the living germ is concerned, but they are most useful in the study of the action of the chemical poison. Our failure to test the action of the germ on this animal thoroughly is due to the fact that our stock of white mice has been very limited during the past year, and we have used the few we did have in other lines of

experimentation. However, two were inoculated subcutaneously with one drop each of an exudate from a rabbit, and were found dead after twelve hours. The post-mortem condition was the same as that observed in guinea-pigs inoculated in the same manner and with like cultures.

Experiments on Dogs. The following will illustrate the action of this bacillus on dogs, and show the differences between the effects of the intensified and the less virulent cultures:

An agar culture was made from one of the last of the series of fifty-one guinea-pigs already referred to. When this culture was eight days old the growth on the surface of the agar was rubbed up with two cubic centimetres of beef-tea and injected into the abdominal cavity of a St. Bernard dog weighing forty pounds. Within one half-hour the animal began to vomit and one hour later to purge. The vomiting and purging continued at intervals of fifteen minutes or longer for twenty-four hours. The retching movement of the abdominal muscles were frequent and powerful. For twenty-four hours longer the animal remained in a condition of collapse, and refused food. After this recovery slowly followed.

A second dog, weighing only eighteen pounds, received an agar culture which had not been intensified by being passed through animals. Twenty-five minutes later the dog vomited once, but further than this it was not affected.

The Poisonous Chemical Products. Our attempts to isolate the chemical poison or poisons of this bacillus have not been successful. However, we have ascertained some facts along this line, and these may be worthy of record.

Certain precautions must be observed by one who attempts to isolate the active, chemical constituents of bacterial cultures. The opportunities for falling into error are many, and the difficulties in the prosecution of the work are often great. We have met with some interesting experiences in this connection. In the first place, we hoped that we had in our new bacillus the generator of tyrotoxicon, and with the idea of determining this we rendered our filtered-milk culture alkaline, shook it with ether, allowed the ether to evaporate spontaneously and injected the residue dissolved in water into animals. The animals thus treated died very speedily, but, recalling the experience that one of us had in his study of tyrotoxicon, we tested the

residue from the ether alone, and found this to be most intensely poisonous. The residue from fifty cubic centimetres of this ether injected subcutaneously or intra-abdominally into a guinea-pig of from three to four hundred grams killed the animal within ten minutes. This ether is the product of a German manufacturer of good reputation. The residue from five hundred cubic centimetres of Squibb's ether produced no effect on animals. We found a recently received lot from another German firm, also free from harmful constituents. With these tested ethers the extractions, to be reported later, were made.

We have observed that some of the animals used in our experiments are very susceptible to the action of alcohol. Special attention must be given to this point in the administration of proteids precipitated by alcohol. These precipitates hold alcohol very persistently when dried in vacuo, and the amount of alcohol thus retained may be sufficiently large to markedly affect rabbits, rats, and guinea-pigs. These proteid precipitates may also, when apparently quite dry, contain enough ether, which we have sometimes used for washing out the alcohol, to affect the animals.

Hydrogen sulphide, when employed for the purpose of removing mercury, platinum, or other base used as a precipitant in cultures, is driven off with great difficulty, and it is a most potent poison to guinea-pigs.

Milk cultures, on account of their complex composition, are notoriously unsuitable for the isolation of bacterial poison. However, as these cultures of this bacillus are especially virulent, we have employed them, with some exceptions, to be stated later. Two-litre flasks, each containing one litre of sterilized milk, were inoculated with the germ taken directly from the peritoneal cavity of a guinea-pig. These flasks were kept in the incubator for thirty days. The contents were then filtered through paper. As soon as the pores of the paper were coated with the proteid part of the culture the filtrate became not only perfectly clear, but sterile. Thus the slow filtration through porcelain, practised in our first experiments, became unnecessary. From five to ten cubic centimetres of this filtrate injected into the abdominal cavity of full-grown white rats or half-grown guinea-pigs caused death within less than one hour. These filtered cultures were then distilled in vacuo at a temperature not above 40°,

until there remained in the retort not more than one hundred cubic centimetres. The distillate was acid with the pleasant odor of the original culture, and ten cubic centimetres failed to induce any symptoms in rats. Five cubic centimetres of the concentrated fluid in the retort killed rats within from five to ten minutes. This concentrated fluid, which was strongly acid, was shaken twice with double its volume of ether. On spontaneous evaporation the ether left a very small residue, which sometimes contained a few imperfect crystals. This residue injected into a full-grown rat killed it within four minutes. The above experiment was repeated many times, and, although the quantity of poison left on the evaporation was found to be variable, it was never altogether wanting. In some instances the residue from the ether consisted of a few drops of a brownish, oily semifluid. In others the residue was perfectly dry, and when examined under the microscope showed some granular matter mixed with a few imperfect and broken prisms. The removal of the poison from the concentrated fluid is imperfect and incomplete, as was shown by driving off the traces of ether from the fluid by keeping it for days in vacuo at 40°, and then injecting some of it into animals, when death resulted quite as promptly as before the extraction with ether was made. When this method was employed with the Uschinsky culture, the amount of the poison left on the evaporation of the ether was much less than that obtained from an equal volume of a milk culture. The animals died, but not until several hours after the injection. We have not been able to obtain enough of the poison to enable us to identify it chemically.

Many other methods of isolation have been attempted, but without success. The distillation was in several instances continued *in vacuo* until only a syrupy residue remained. This residue was extracted with absolute alcohol, which dissolves the poison, the alcoholic extract was evaporated, and this residue again treated with absolute alcohol. This was repeated as many as a dozen times and the alcoholic solution was finally precipitated with platinum chloride. This precipitate was crystallized, but was found to consist of a sodium salt.

In another experiment, the residue obtained after repeated extractions with alcohol was distilled *in vacuo* at a high temperature. At 130° a clear fluid passed over, but this consisted of glycerin containing only traces of the poison which was demonstrated by its action on animals, while the residue in the retort was found to be inert.

From the concentrated, filtered culture, when made alkaline with either ammonia or a fixed alkali, the poison is not removed by ether. This distinguishes this poison chemically from tyrotoxicon. Physiologically this poison is distinguished from tyrotoxicon by the more pronounced effect of the former on the heart, in which it resembles muscarin or neurin more closely than it does tyrotoxicon. Anatomically the two are unlike, inasmuch as the product of our bacillus induces marked congestion of the tissues about the point of injection or in the peritoneum when thrown into the abdominal cavity. Moreover, the intestinal constriction which was so universally observed in animals poisoned with tyrotoxicon has not been once seen in our work with this new germ and its poison, although it has been carefully looked for in the more than two hundred animals experimented with.

The poison is not removed from either acid or alkaline solutions with chloroform.

The following experiment was made in order to determine whether or not our bacillus elaborates a proteid poison or a toxin which is precipitated with the proteids. For this purpose an Uschinsky culture was selected, inasmuch as such a culture contains no proteids save those elaborated by the germ. A litre of an Uschinsky culture forty days old was filtered through porcelain in order to remove the germs. The clear, strongly acid filtrate was allowed to fall, drop by drop, into twice its volume of absolute alcohol. A flocculent, white preciptate fell and formed a thin layer on the bottom of the cylinder. This precipitate was collected on a filter and washed for two days with absolute alcohol. It was then dried between folds of filter paper and rubbed to a powder in an agate mortar. Twenty milligrams of this powder were suspended in water and injected into the abdominal cavity of a guinea-pig. The animal showed no effect of the poison at the time, but it died two days later. Post-mortem examination showed the same condition as had been observed after death from inoculation with the bacillus and after death from the poison extracted with ether. The peritoneum was highly congested, the abdominal cavity contained a reddish exudate, and the heart was in diastole and filled with blood. It may be that enough of the same poison, which is extracted with ether, had been carried down mechanically with a non-poisonous proteid, and had not been removed by the repeated washings with alcohol. If this be the case, or if there be two chemically distinct poisons, we

are not able to determine at present. The Uschinsky fluid which had been treated with two volumes of absolute alcohol, and from which the alcoholic precipitate had been removed as just stated, was concentrated *in vacuo* and the concentrated fluid was shaken with two volumes of ether, and the residue left on the evaporation of the ether injected into a guinea-pig caused death within four hours. Post-mortem examination showed the condition already described as due to the germ and the germ-free cultures.

By a mistake, in which a germ-free milk culture reduced to about half its volume was placed in a bottle labelled nucleinic acid, ten minims of this fluid were injected subcutaneously into a patient of about one hundred and fifty pounds weight. Within thirty minutes this person began to complain of dizziness. A few minutes later there was free vomiting. A little later the bowels moved freely. The vomiting and purging continued at intervals of a few minutes for four or five hours. Two hours after the injection the patient complained of deafness and responded only when the lips were placed near the ear and the words spoken in a loud voice. A little later there was wild delirium, the patient constantly attempting to get out of bed. Three hours after the injection the patient fell into a comatose condition. The feet and hands were cold and the radial pulse was imperceptible. Strychnin was given hypodermically and recovery followed slowly but completely.

Twelve hours after the injection all alarming symptoms had subsided, but it was two days before the patient was able to walk about the room. The next day the action of this fluid on guinea-pigs was carefully and repeatedly tested. The animals selected for this purpose weighed from one hundred and ninety to two hundred grams. Ten minims injected subcutaneously had no visible effect on them. One cubic centimetre induced only a slight and temporary effect. Within from five to ten minutes after such injections the animal retched a few times, but manifested no further symptoms. Two cubic centimetres caused death within from four to eight hours. These facts illustrate the difference in susceptibility to the action of this poison in man and guinea-pigs. Evidently our bacillus elaborates a poison of most potent action in man.

In our studies of the chemical poison we have evaporated the germ-free cultures *in vacuo* and at a low temperature. It must not be

inferred from this that higher temperatures quickly decompose the poison. Cultures heated in open dishes on the water-bath for hours, with the contents of the dish at 80° to 90°, do not lose their toxicity, and even brisk boiling over the naked flame for fifteen minutes is without appreciable effect. This shows that milk containing this poison, even after sterilization by heat, may not be altogether harmless. However, as we have already seen the germ is killed at comparatively a low temperature and by the sterilization of milk the further elaboration of the poison is prevented.